ON A NEW RHIZOPOD PARASITE OF MAN (AMŒBA MIURAI N. SP.)

By Prof. I. IJIMA, Ph. D.

The Rhizopod parasite of man, reported on in this paper, apparently represents a hitherto undescribed species of Amœbæa. I take pleasure in proposing to call it *Amœba miurai* after Prof. K. MIURA of the Medical College, Imperial University of Tokyo, who first discovered it and kindly handed over to me the materials for study and description. The patient, who harbored the parasite, was a married woman, Yuki Ishiwatari by name and twenty-six years of age. She resided in the Prefecture of Kanagawa until she was taken into the University Hospital at the end of November last year. Her disease consisted at first in abdominal tumors which could be felt from outside andin ascites-like accumulation of fluid in the abdominal cavity. Later the affliction increased the degree of malignance and extended itself into the left pleural cavity. The patient finally succumbed in August of the present year. As the result of clinical and post mortem examinations Prof. MIURA has arrived at the conclusion, that he had to do with a case of *peritonitis* and *pleuritis endotheliomatosa*. For the details of this case from medical standpoint I refer those interested to a forthcoming paper of Prof. MIURA himself, which will appear in the "Mittheilungen aus der medicinischen Facultät der Kaiserlichen Universität zu Tokyo."

It was in the serous fluid-accumulation of the peritoneal as well as of the pleural cavity that the Amœbæ were found in abundance. Only during a period of about two days, shortly before the patient's death, they were also present in the fæces concomitantly with hæmorrhage in the intestine; at other times the fæces were free of them.

The discovery reminded us at once of *Leydenia gemmipara* Schaudinn, a human parasitic Rhizopod discovered two years ago in Berlin under almost identical circumstances.* However, it was evident without going into discriminating

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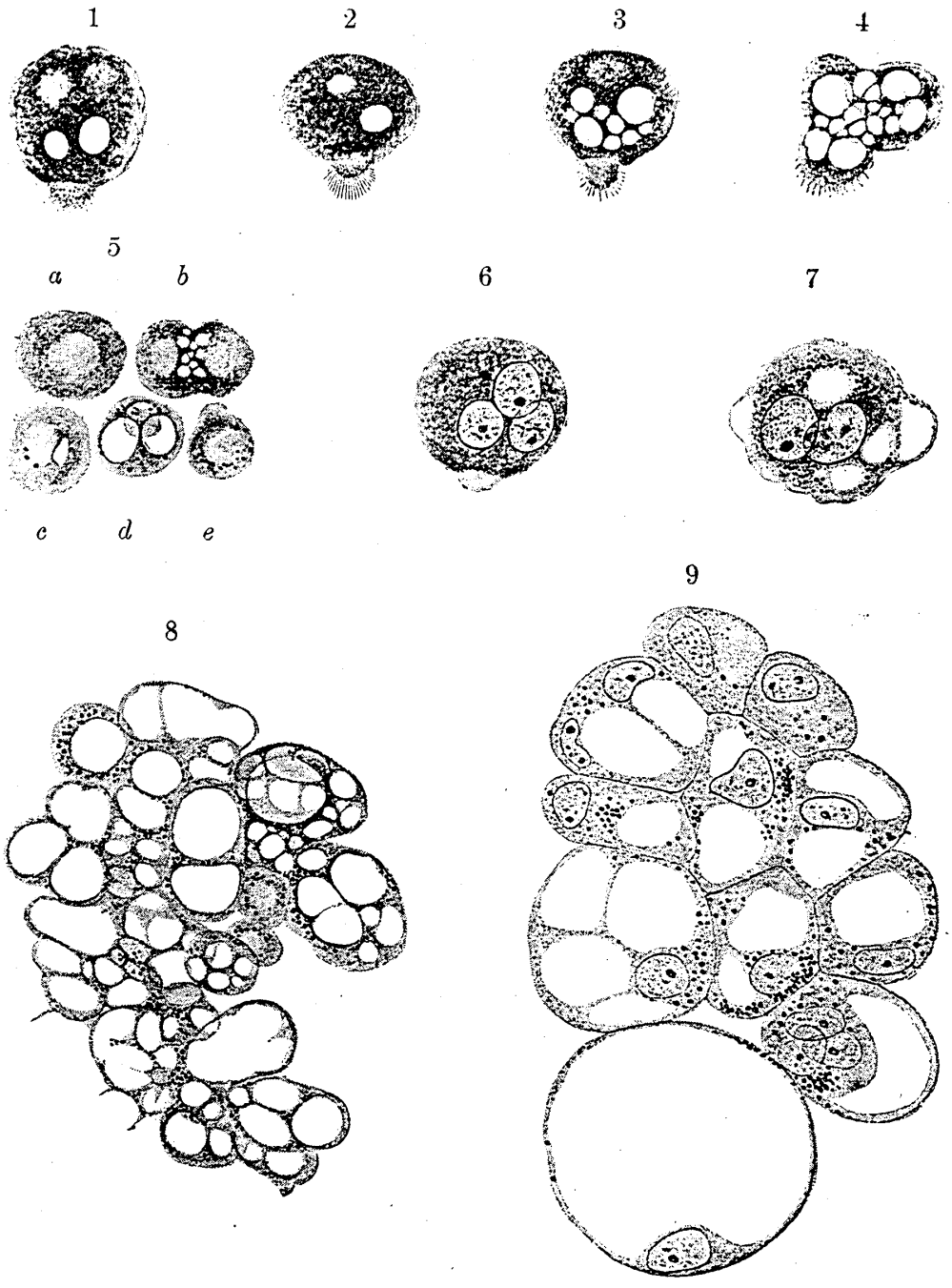
* v. Leyden and Schaudinn: Sitz ber. d. k pr. Akad. d Wiss. Berlin, 1893, XXXIX.



Amœba miurai Ij.

All figures magnified 500 times. Figs. 1—5 and 8 drawn from fresh state; figs. 6, 7 and 9, after treatment, with acetic acid.

Fig. 1.—A living specimen. with the surface in slow wave-like motion. Below, the villous knob beset with short pseudopodia. Internally, two vacuoles with sharp contour, two nuclei represented by ill-defined clear spaces and a few oil-like corpuscles.

comparisons that the present Amœba represents a form quite distinct from the one just mentioned.

The serous fluid, obtained from time to time by repeated punctures, was always of the same nature and appearance, well agreeing with v. LEYDEN's account (loc. cit.) of the ascites-fluid in which *Leydenia gemmipara* was found. It was of a dark-reddish color on account of a large proportion of the blood it contained. When left standing for a few hours in a vessel, it separated into a serum f yellowish colour with greenish fluorescence and a thick sediment of dark-red color. The latter, when examined under the microscope, revealed the usual elements of a blood-coagulum (red and white blood-corpuscles, fibrin net-work) besides a variable number of what appeared to be endothelial cells in the process of fatty degeneration and a multitude of peculiar bodies, the Amœba to be presently described (*vide* annexed half-tone cuts).

These were by no means uniform in appearance. While some showed a very characteristic shape and were evidently alive, others were abnormally vacuolated more or less swollen and apparently dead or nearly so. It is a noteworthy fact that both dead and living specimens were found together even in the fluid examined on warm stage immediately after extraction.

Individuals in living and consequently normal state (figs. 1—3) were found always isolated, never adhering together in clusters. The body of such is habi-

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Fig. 2.—Same as above, the pseudopodia on the knob extended filament-like. Nucleus not visible. Vacuoles and oil-like corpuscles as in fig. 1.

Fig. 3.—Another living specimen, in which the villous knob is bounded against the main body by a shallow ring-groove. Several vacuoles within; above these the nucleus is indicated by the clearer appearance of the sarcode.

Fig. 4.—A fresh specimen in the first stage of becoming morbid, but still showing some pseud podia. The knob bearing the latter is being encroached upon by the vacuoles which are enlarging themselves by imbibition.

Fig. 5.—Small, probably young specimens. *a*, with uneven surface; neither vacuoles nor oil-like corpuscles present, but with nucleus indistinctly recognizable at centre. *b*, biscuit-shaped and the two nuclei so disposed as if in process of cell-division; several small vacuoles and a few oil-like corpuscles at the middle. *c*, the surface uneven and with pseudopodia-like processes; a single small vacuole and a scanty number of oil-like corpuscles present. *d*, spherical, with three vacuoles and a fair number of oil-like corpuscles In all the above figures the villous knob is either concealed from view or not developed at all. *e*, with unmistakeable knob but without villi or pseudopodia; no vacuole; numerous oil-like corpuscles around the nucleus.

Fig. 6.—A specimen treated with dilute acetic acid solution. Pseudopodia on the knob retracted; three nuclei made distinctly visible; no vacuole.

Fig. 7.—A similarly treated specimen with two nuclei. The knob is either concealed or obliterated. The vacuoles have lost sharpness of contour. The accumulation of imbibed fluid has caused the pellicle to heave up, pustule-like, at several places.

Fig. 8.—Portion of a large mass formed by the cohering together of dead, strongly vacuolated individuals. Seen in fresh state.

Fig. 9.—A similar cluster of dead individuals, seen after treatment with acetic acid (greatly swollen but nuclei made distinct).

tually spherical or more frequently ellipsoidal, characterized by having at one pole a small rounded protuberance, which on close observation is found to bear on its surface a number of fine processes, the pseudopodia, closely set and extended to a greater or less degree. The protuberance is apparently the same structure as the "villous knob" or "Zottenanhang" which has long been known to characterize the hind end of certain species of Amœbæa (*Amœba villosa* Wallich, *Am. fluida* Gruber, *Pelomyxa villosa* Leidy).*

The size of the body is variable within certain limits. Large specimens have a diameter of 38 μ, while the smallest may measure not more than 15 μ across. They never attain the size of *Am. fluida* (80—90 μ, according to GREEFF), which, of all the Amœba species known to me seems to come nearest to the present one in several respects.

The sarcode is, apart from its enclosures, clear and uniformly finely granular, without perceptible differentiation into the ectoplasm and the endoplasm except at the villous knob. The substance of the latter is clearer and hyaline, without enclosures of any kind. I think I may say that it represents the main, if not the entire, mass of the ectoplasm of the present species, localized as it were at the spot in questien. This view also coincides with the fact that the general surface never involves itself in any considerable movements.

The villous knob may be papilliform or hemispherical in shape, measuring about 10 μ across at the base. At other times it is only a gentle elevation and under certain circumstances, may even be entirely retracted or obliterated. On small specimens, such as represented in fig. 5 *a*—*d*, I have frequently missed the knob. It is possible that in some of such cases it was simply concealed from view, being situated at a position turned away from or towards the sight; in certain other cases however I was convinced of its absence. In these latter cases, short

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* *Am. villosa* is a fresh-water species first discovered and described by WALLICH in England (Ann. & Mag. Nat. Hist. 1863). Whether the forms reported under the same name by LEIDY (Fresh-water Rhizopods of N. America, 1879) and by MÖBIUS (Rhizopodenfauna der Kieler Bucht; Abh. d. k. pr. Akad. d. Wiss. zu Berlin, 1888) were correctly identified, seems to require corroboration.—*Am. fluida* is a marine species first described by GRUBER (Z. f. w. Z. Bd. 41, p. 219) and later more precisely by GREEFF (Biol. Centralbl. Bd. 12, p. 374). This is a species that seems most to resemble *Am. miurai*.—*Pelomyxa villosa* was described by LEIDY in his "Fresh-water Rhizopods of N. America," p 75.—All these species have in common with *Am. miurai* the characteristic villous knob, though it can not be said that this structure is strictly confined to the species mentioned. As to the specific distinction between *Am miurai* and the three species above cited let it suffice to mention here that the latter are all much larger in size, are capable of active, typically amœboid or flowing motion with the main body and inclose in the endosarc crystals, pigments or peculiar bodies such as are not observed in *Am. miurai*.

villi-like pseudopodia were sometimes found emanating in irregular distribution from the general surface (fig. 5, *a* & *c*), what might be expected to occur in immature individuals so long as these would be naked and the ectoplasm not concentrated into a knob.

It sometimes happens in life that the villiform pseudopodia are entirely retracted. The knob then presents a smooth surface (fig. 5, *e*), as it does always when acted upon by reagents (fig. 6). Otherwise it is beset with shorter or longer pseudopodia as the case may be. When short, the pseudopodia are generally conical in shape and comparatively thick though minute (fig. 1). By focussing up and down the microscope, it was easy to observe the knob-surface closely studded over with them. When fully extended (fig. 2), they may reach 5 μ in length and are extremely fine, broadest at base and thinned out towards end. They then seem to radiate forth in tolerably straight course. Although I do not remember having ever seen them branch or anastomose, yet I do not feel myself sufficiently warranted to exclude the possibility of such occurrence. The actual movement of the pseudopodia, whether molecular or otherwise, could not be watched in continuous succession, what is sufficiently accounted for by the slowness of motion combined with the fineness and the hyaline nature of the pseudopodia. On the other hand, by examining the same living object at intervals of several minutes, I could plainly observe, under favorable circumstances, the variation in the degree of contraction or elongation of the filamentous structures under consideration,— evidence enough that these are to be seen in the light of pseudopodia and not of immobile villi. The same view has been put forward by GREEFF* for the identical structures of *Am. fluida*.

The so-called villous knob passes usually, though not always, insensibly into the main body. Not unfrequently, however, there were cases in which the two parts were separated externally by a tolerably sharp line of demarcation (fig. 3). This was brought about by the presence of a shallow ring-groove surrounding the basis of the knob. The appearance then is as if either the knob-base has just slightly sunk into the main body or the latter has elevated itself in a low wall around the former. This is without doubt only a temporary condition arising from a certain state of contraction of the sarcode.

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* GREEFF: Biol. Centralbl. Bd. XII, p. 377.

To all appearance the villous knob is naked, i.e., devoid of an external enveloping membrane The same can not be said of the main body. An indubitable, double-contoured membrane is indeed not directly demonstrable in either fresh or prepared specimens under ordinary circumstances. The contour-line of the main body appears sharp but is simple. Nevertheless, it often happens after death that the entire Amœba is blown out into a thin-walled vesicle by the excessive enlargement of the vacuoles contained, then to remain in that state for a considerable length of time, giving an impression as if the tension of a special superficial layer resisted its speedy bursting. Again, should the animal be left for some time in a dilute solution of acetic acid, the fluid imbibed into the sarcode frequently accumulates itself in the form of vacuoles just under the surface and heaves up from below a pellicle in a pustule-like manner (fig. 7). These appearances have led me to infer that a thin elastic layer of a firmer consistency than the internal sarcode covers the whole surface, interrupted only at one spot by an opening through which the pseudopodia-producing ectoplasm is protruded knob-like into the exterior. This would be exactly the same state of things as has been described in certain near allies of the present species, e. g., in *Amœba fluida* by GREEFF,* in the genus *Plagiophrys* by ARCHER† and PENARD‡, a condition that leads over to that seen in the soft-shelled, monothalamous and monostomatous Rhizopods. In *Am. fluida* the membrane should be thicker and more distinct than in the present species.

The main body is not altogether incapable of changing its form but unlike its known nearest allies *Am. villosa* and *Am. fluida*, the motion is so slow and limited in extent that it requires close observation to perceive it. The shape may change from spherical into ellipsoidal or *vice versâ* and at times assume a somewhat irregular outline. In one case I have observed a slow wave-like movement of the surface, so that the latter presented a slightly verrucose appearance (fig. 1). A "flowing" motion of the sarcode or such active transformation of the body into lobate pseudopodia as is ascribed to *Am. villosa* or *Am. fluida*, was never noticed. On the contrary, the various enclosures retained tolerably constant relative positions all the while during observation. It seemed as if the

* GREEFF: loc. cit., p. 375.
† ARCHER: Quart. Jour. Micr. Sci. Vol. XI.
‡ PENARD: Mém. Soc. Physiq. H. N. Genève, T. XXXI, No. 2.

sarcode were not of sufficiently fluid nature as to allow of a far-reaching change in the body-form. So then, *Am. miurai* must be said as being of a very sluggish habit. On this account and from consideration of certain fact to be mentioned further on but which indicated that the Amœba was not fit for prolonged existence in the serous fluid containing it, I have naturally questioned myself if the forms I have considered as normal and healthy were really such and not already in the first stage of contraction. But I think this doubt can be done away with as being unfounded, for, were the animal in any way pathologically affected, the power of emitting and retracting those delicate pseudopodia on the knob should be the first to disappear.

The enclosures in the main body are the nucleus, the vacuoles and the minute oil-like corpuscles. They occur in the finely granular sarcode without any definite rule as to their positions.

The nucleus is generally invisible in the fresh or living state, at most only indistinctly indicated by an ill-defined, somewhat clearer space in the sarcode (figs. 1—5). When treated with the acetic acid, it comes forth with all the desirable distinctness (figs. 6,7 & 9). It occurs in twos or threes almost as often as it does in a single number. Round, oval or kidney-like in shape, it is bounded by a distinct nuclear membrane. The diameter measures 8—15 μ. The nuclear fluid is faintly granular, somewhat clearer than the sarcode and encloses within one or more prominent nucleoli, generally one in number.

The vacuoles are perfectly clear and form very conspicuous objects in fresh specimens, being very sharply outlined against the sarcode. They are inconstant as to their number and size. In some, notably smaller, individuals (fig. 5, *a* & *e*; fig. 6), they were found to be even entirely missing. But the majority of individuals showed them in numbers of one, two, three or several (see figs.). I think none of these vacuoles is pulsatile. Once a vacuole in a specimen, the first examined from a freshly taken abdominal fluid, was seen to vanish from view as slowly as it again reappeared; but then I was at a loss to decide, whether or not, the phenomenon was simply due to that vacuole getting alternately into and out of the focus as the object slowly rolled about under the cover-glass. Treated with acetic acid, the vacuoles lose the boldness of contour, while the large vesicular nucleus, hitherto concealed, is made perfectly clear. In the number, size and non-contractility of the vacuoles the present species seems to agree exactly with *Am. fluida* as described by GREEFF.

The oil-like corpuscles are small yellowish, highly refractive spherules of by no means uniform size. They are probably nutritive matter in reserve and identical with similar bodies that are so commonly met with in the body of other Amœbæ. Some individuals contained only a small number of these corpuscles, others in fair abundance. Also cases were not wanting in which not a trace of them was to be found.

Crystals and extrinsic matter, such as food-particles, &c., were not met with in the sarcode. Nor was the animal ever seen in the act of taking in food, which process, in my opinion, could only take place by means of the villiform pseudopodia at the knob. Whether the latter, like the similar organ of *Pelomyxa*, served at times for prehension, I have not been able to ascertain.

The above is the description of *Amœba miurai* in what I consider its normal living state. Now besides such individuals, the serous fluid also contained a large quantity of peculiar cells, which were unmistakably nothing else than dead, at any rate much changed, bodies of the same animal. These are usually globular or more or less irregular in shape and of about the same size as normal individuals or larger on account of their swollen state. They are found either isolated or clinging together in variable numbers and forming conglomerate-like clusters (figs. 8 & 9). Sometimes such clusters are as large as to present a dimension of almost half a millimeter. The cells are characterized by having one or several large vacuoles that press the scanty protoplasm and the nucleus between them or against the peripheral wall. They often present the form of thin-walled strongly distended vesicles. The protoplasm contains the same oil-like corpuscles as the normal specimens; the nuclei, made visible after treatment with acetic acid, are likewise exactly the same. The villous knob and with it the pseudopodia have disappeared, leaving no trace whatever. A similar swelling was observed by GREEFF in *Am. fluida* when left in certain liquids, the enveloping membrane then showing a gap at the position where the villous knob has disappeared. Such a gap was not visible in my objects, what was probably largely due to the thinness of the membrane. The cells have not the slightest power of active motion and I think no one, who sees them, will hesitate to consider them as dead and as being prevented from speedy bursting and collapse only by the presence of an enveloping membrane. The existence of transitional stages between the normally

conditioned *Amœba* and the cells in question definitely establishes the derivation of the latter from the former. In fig. 4 is represented a specimen which is evidently on the verge of becoming morbid. It still shows signs of life inasmuch as it possesses some pseudopodia, but the knob is stretched out to a great extent by the vacuoles that are encroaching upon it. Indication is not wanting that a part of the swelling contents has protruded itself hernia-like through the opening of the enveloping membrane. Should the pseudopodia in such a specimen cease to exist with the extinction of life and the body swell somewhat more as the result of imbibition, the metamorphosis into the state of the above described cells would be completed.

As already mentioned, both the living and the dead individuals were found together even in the freshest fluid, still warm and guarded against injurious influences. Care was taken to sterilize all the wares and instruments that were to come into contact with the fluid and observations were made by means of a microscope to which was fixed an arrangement that effectually kept the preparation at the normal body-temperature. Examined under such precautions, every preparation made of a drop of the fluid always contained the *Amœba* in the two conditions referred to, in such a number that it hardly ever needed a much prolonged search to come across one or the other kind, even though the power used were a moderately strong one. Preparations of the sediment, that formed itself after standing for some time, of course contained the parasites in much larger proportions, the majority of which were dead and adhering together in clusters. In the fluid kept overnight, they were almost all, if not without exception, dead and much swollen up. It is important to mention that it made no difference on their mortality whether the fluid was allowed to cool or kept in a warm chamber at the body-temperature ever since its extraction. Let it be also mentioned here that I have not been able to observe the mode of reproduction, beyond what is suggested by the possession of more than one nuclei or the occasional occurrence of biscuit-like forms (fig. 5, *b*).

The above observation tends to show, in my opinion, that the serous fluid was not a medium fit for the parasite to continue its vigorous existence,—that the real place of its parasitism is to be sought somewhere else than in the fluid that contained it. The parasites were evidently dying off in the serous fluid while

still within the patient's body-cavity. Above all then, the tissues of the tumors suggest themselves as most likely the proper home of the parasites, whence they might have fallen into the fluid-accumulation of the body-cavity or into the alimentary canal by rupture. At the post mortem examination, which was undertaken eighteen hours after death, Prof. MIURA found a number of dead, swollen and motionless Amœbæ on the surface of the tumors. Further results of his extended researches on the pathological parts remain yet unknown to me. It is hoped that in his forthcoming report he will be able to bring forward facts which will help to clear up the question of the relation that the parasites bore to the patient's disease.

In conclusion I wish to express my thanks to Prof. K. MIURA for supplying me with both materials and informations, without which I could never have been able to complete this report.

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